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FIG. 1

```
cons.aa                   G  G  G  V                    A  K                E
hTGFBR-II    LDTLVGKGRFAEVYKAKLKQNTSEQPETVAVKIFPYDHYASWKDRKDIFSDINLKHENILQF
mActR-IIB    LLEIKARGRFGCVWKAQLMN------DFVAVKIFPLQDKQSWQSEREIFSTPGMKHENLLQF
mActR-II     LLEVKARGRFGCVWKAQLLN------EYVAVKIFPIQDKQSWQNEYEVYSLPGMKHENLLQF
daf-1        LTGRVGSGRFGNVSRGDYRG------EAVAVKVFNAIDEPAFHKEIEIFETRMLRHPNVLRY
subdomains          I                    II           III              IV


hTGFBR-II    LTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRNVGGSLARGLSHLHSDHTP-C
mActR-IIB    IAAEKRGSNLEVELMLITAFHDKGSLIDYLKGNIITWNELCHVAETMSRGISYLHEDVPWCR
mActR-II     IGAEKRGTSVDVDLMLITAFHEKGSLSDFLKANVVSWNELCHIAETMARGLAYLHEDIPGLK
daf-1        IGSDRVDTGFVTELMLVIEYHPSGSLHDFLLENTVNIETYYNLMRSTASGLAFLHNQIGGSK
subdomains                V                                        VI-A


cons.aa                       DLK    N              DFG
hTGFBR-II    -GRPKMPIVHRDLKSSNILVKNDLTCCLCDFGLSLRL---GPYSSVDDLANSGQVGTARYMAP
mActR-IIB    GEGHKPSIAHRDFKSKNVLLKSDLTAVLADFGLAVRF---EPGKPPGD--THGQVGTRRYMAP
mActR-II     -DGHKPAISHRDIKSKNVLLKNNLTACIADFGLALKF---EAGKSAGD--THGQVGTRRYMAP
daf-1        -ESNKPAMAHRDIKSKNIMYKNDLTCAIGDLGLSLSKPEDAASDIIAN--ENYKCGTVRYLAP
subdomains       VI-B                         VII                      VIII
```

1

FIG. 2A

```
a.a          C   C   E   G   N   M   C
5' GCGGATCCTGTTGTGAAGGNAATATGTG 3'
        BAMHI   C   C   G       C
```

FIG. 2B

```
a.a         V   A   V   K   I F
5' GCGGATCCGTCGCAGTCAAAATTTT 3'
   BamHI        G   C   G   G   C
               T   T   T       A
```

FIG. 2C

```
a.a       R   D   I K   S   K N
5' GCGGATCCGCGATATTAAAAGCAA 3'
      BAMHI    A   C   C   GTCT
               G       A
```

FIG. 2D

```
a.a         E   P   A   M   Y
5' CGGAATTCTGGTGCCATATA
      EcoRI G   G       G
            A   A
```

2

FIG. 3A

```
MGAAAKLAFAVFLISCSSGAILGR          ActR-II
  MTAPWAALLALLWGSLCAGSGRGE         ActR-IIB
       MEAAVAAPRPRLLLVLAAAA        TβR-II
            MTLGSPRKGLLMLLMALV     TβR-I/ALK-5
            MVDGVMILPVLIMIALPSP    ALK-1
                                   ALK-2
       MAESAGASSFPLVVLLL           ALK-3
                                   ALK-4
            MLLRSSGKLNVGTKKE       ALK-6
```

```
MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAV

                                    •
SETQECLFFNANWEKDRTNQTGVEPCYGDK--DKRRH-CFATWKN   ActR-II
AETRECIYYNANWELERTNQSGLERCEGEQ--DKRLH-CYASWRN   ActR-IIB
KFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK   TβR-II
AAAAALLPGATALQCFCHL-CTKD-NFTCVTDGL-CFVSVTE      TβR-I/ALK-5
TQGDPVKPSRGPLVTCTCESPHC-KGP--W---CTVVLVR        ALK-1
SMEDEKPKVNPKLYMCVCEGLSCGNED---HCEGQ-Q--CFSSLSI  ALK-2
NGVTLAPEDTLPFLKCYCSG-HCPDDAINNTCITNGH-CFAIIEE   ALK-3
AGSGSGPRGVQALLCACTS--CLQA-NYTCETDGA-CMVSIFN     ALK-4
DGESTAPTPRPKILRCKCHH-HCPEDSVNNICSTDGY-CFTMIEE   ALK-6
```

```
MTQLYIYIRLLGAYLFIISRVQGQNLDSMLHGTGMKSDSDQKKSE

                                    •
ISGSIEIVKQGCWLDDINCYD---RTDCV--EKKDSPEVYF       ActR-II
SSGTIELVKKGCWLDDFNCYD----RQECV-ATEENPQVYF       ActR-IIB
NDENITL-ETVCHDPKLPYHDFILEDAASPKCIMKEKKPGETFF    TβR-II
TT-DKVIHNSMC-IAEID----LIPRDRPFVCAPSSKTGSVTTTY   TβR-I/ALK-5
EEGRHPQEHRGC---GN-----LHRE--LCRGRPTEFV--NHY     ALK-1
NDGFHVY-QKGC-FQ----VYEQGKMTCKTPPSPGQ--AVE       ALK-2
DDQGETTLASGC-MKY-----EGSDFQCKAQLRRTIE           ALK-3
LD-GMEHHVRTC-IPKVE---LVPAGKPFYCLSSED--LRNTH     ALK-4
DDSGMPVVTSGC-LGL-----EGSDFQCRDTEPIPHQRRSIE      ALK-6
```

FIG. 3B

A multiple sequence alignment of type I and type II serine/threonine kinase receptors, shown in three stacked blocks. Each block is labeled on the right with the receptor names:

- ActR-II
- ActR-IIB
- TβR-II
- TβR-I/ALK-5
- ALK-1
- ALK-2
- ALK-3
- ALK-4
- ALK-6

FIG. 3C

Protein kinase domain sequence alignment for: ActR-II, ActR-IIB, TβR-II, TβR-I/ALK-5, ALK-1, ALK-2, ALK-3, ALK-4, ALK-6

Block 1 (regions I, II)

```
FGCVWKAQLLN - - - - - EYVAVKIFPIQDKQSWQNEYEVYSLPGM   ActR-II
FGCVWKAQLMN - - - - - DFVAVKIFPLQDKQSWQSEREIFSTPGM   ActR-IIB
FAEVYKAKLKQNTSEQFET - VAVKIFPYEEYASWKTEKDIFSDINL   TβR-II
FGEVWRGKWRGEE - - - - - VAVKIFSSRDEQSWFRETEIYQTVLL   TβR-I/ALK-5
YGEVWRGLWHGES - - - - - VAVKIFSSRDEKSWFRETEIYNTVML   ALK-1
YGEVWRGSWQGEN - - - - - VAVKVFFTTEEASWFRETEIYQTVML   ALK-2
YGEVWMGKWRGER - - - - - VAVKVFFTTEEASWFRETEIYQTVLM   ALK-3
FGEVWRGRWRGGD - - - - - VAVKVFFTTEEASWFRETEIYQTVML   ALK-4
YGEVWMGKWRGEK - - - - - VAVKVFFTTEEASWFRETEIYQTVLM   ALK-6
                              I            II        III
```

Block 2 (regions IV, V)

```
KHENILQFIGAEKRGTSVDVDLWLITAFHEKGSLSDFLKANVVSW   ActR-II
KHENILQFIAAEKRGSNLEVELWLITAFHDKGSLTDYLKGNIITW   ActR-IIB
KHENILGFIAAEKRKELGKQYWLISDYHEHGSLFDYLNRYTVSW    TβR-II
RHDNILGFIASDMTSRNSSTQLWLITHYHEHGSLYDYLQLTTLDT   TβR-I/ALK-5
RHENILGFIASDMTSRHSSTQLWLITHYHEMGSLYDYLQLTTLDT   ALK-1
RHENILGFIAADIKGTGSWTQLYLITDYHENGSLFDYLNRKCATLDT  ALK-2
RHENILGFIAADNKDNGTWTQLWLVSDYHENGSLFDYLNRYTTDT   ALK-3
RHENILGFIAADNKDNGTWTQLWLVSDYHENGSLYDYLNRYSTTDT   ALK-4
RHENILGFIAADIKGTGSWTQLYLITDYHENGSLYDYLKSTTLDA   ALK-6
         IV                         V
```

Block 3 (regions VIA, VIB)

```
NELCHIAETMARGLAYLHEDIP - GLKDGHKPAISHRDIKSKNVLL   ActR-II
NELCHVAETMSRGLSYLHEDVPWCRGRPKPSIAHRDFKSKNVLL    ActR-IIB
EDLRKLGSLLARGIAHLHSDHTPC - - GRPKMPIVHRDLKSSNILV   TβR-II
HLALRLAVSAAGGLAHLHVEI - - - - - VGTQGKPAIAHRDFKSRNVLV  TβR-I/ALK-5
VSCLRLIVLSIASGLAHLHIEI - - - - - FGTQGKPAIAHRDLKSKNILV  ALK-1
RALLKLAYSAAGGLCHLHTEI - - - - - YGTQGKPAIAHRDLKSKNILV  ALK-2
EGMIKLALSAAAGGLCHLHMEI - - - - - VGTQGKPGIAHRDLKSKNILV  ALK-3
KSMLKLAYSSVSGLCHLHTEI - - - - - FSTQGKPAIAHRDLKSKNILV  ALK-4
                                                 VIB
   VIA
```

FIG. 3D

Protein sequence alignment (regions VII, VIII, IX, X) for receptors:

ActR-II, ActR-IIB, TβR-II, TβR-I/ALK-5, ALK-1, ALK-2, ALK-3, ALK-4, ALK-6

FIG. 3E

```
DHDAEARLSAGCVGERITQMQRLTNIITTEDIVTVVTMVTNVDFP   ActR-II
DHDAEARLSAGCVVEERVSLIRRSVNGTTSDCLVSLVTSVTNVDLL  ActR-IIB
DHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKIPEDGSLNTT   TβR-II
YANGAARLTALRIKKTLSQLSQQEGIKM(503)               TβR-I/ALK-5
YPNPSARLTALRIKKTLQKISNSPEKPKVIQ(503)            ALK-1
YQNPSARLTALRIKKTLTKKIDNSLDKLKTDC(509)           ALK-2
AHNPASRLTALRIKKTLAKMVESQDKI(532)                ALK-3
YANGAARLTALRIKKTLSQLSVQEDVKI(505)               ALK-4
AQNPASRLTALRVKKTLAKMSESQDIKL(502)               ALK-6
```

XI

```
PKESSL (513)   ActR-II
PKESSI (536)   ActR-IIB
K      (567)   TβR-II
```
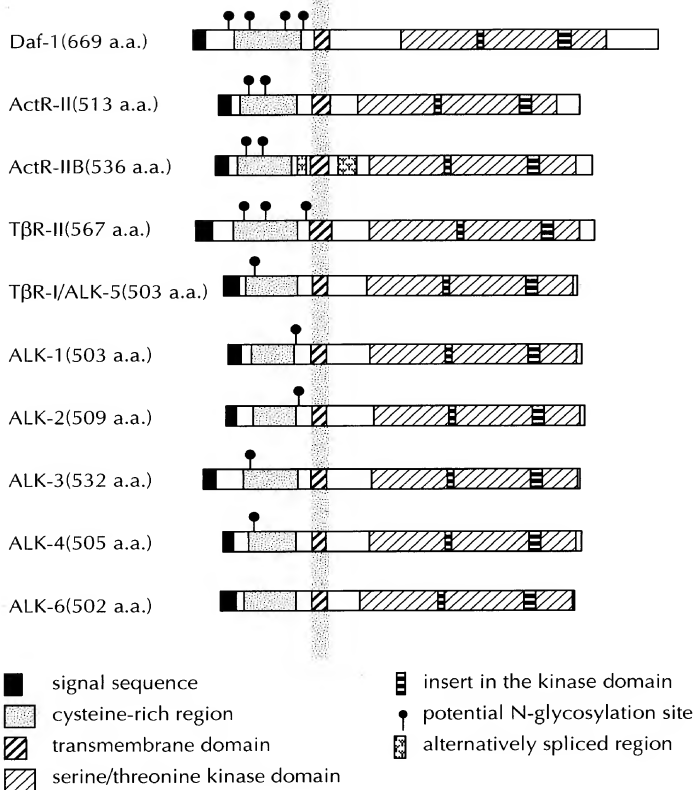
Daf-1(669 a.a.)

ActR-II(513 a.a.)

ActR-IIB(536 a.a.)

TβR-II(567 a.a.)

TβR-I/ALK-5(503 a.a.)

ALK-1(503 a.a.)

ALK-2(509 a.a.)

ALK-3(532 a.a.)

ALK-4(505 a.a.)

ALK-6(502 a.a.)

■ signal sequence

▦ cysteine-rich region

▨ transmembrane domain

▨ serine/threonine kinase domain

▤ insert in the kinase domain

• potential N-glycosylation site

▩ alternatively spliced region

FIG. 4

FIG. 5

```
                              --------------CXGD-DI--TCET--G-CFVSL--SDG   Majority

C-F-------------C---------------------HCKGP-----TCR--GAWCTVLVREEG        AKL-1/CR
C-T------------CESP-----------CEGL-----SCGNEDHCEGQQ--CFSSLSINDG          AKL-2/CR
C-I------------V--------------HCPDD-AINNTCIT-NGHCFAIIEEDDQ               AKL-3/CR
C-Y------------CSG-----------CLQA-NY--TCET-DGACMVSFFNLDG                 AKL-4/CR
C-A------------CTS-----------CTKD-NF--TCVI-DGLCFVSVTETTD                 AKL-5/CR
C-F------------CHL-----ANWEKDRTNQTGVEPCYGDKDKRRHCFAT---WKNI--SIGS        ActR-II/CR
CLEFN-----------------ANWELERTNQSGLERCEGEQDKRLHCYAS---WRNS-SIGT          ActR-IIB/CR
CIYYN--------CDVRFST----CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDE                TβR-II/CR
CKFJ---CDVRFST-CNARTTGWVPGIEFLNETDRSFYENT--CYID-GSCYQSA--RPS             DAF-1/CR
HCSREVGC

                    IEIVEKGC------CYDRTL---GSPF-CVKSPKSPG-TVTEC-CEGDLC    Majority

RHPQEHRG------CGNLH----REL-CRGRPTE-FVNHYC-CDSHLC                         AKL-1/CR
FHVYQKGC------FQVY---EQGKMTC--KTPPSPGQAV-EC-CQGDWC                       AKL-2/CR
GETTLASG------CMKYE---GSDFQCKDSPKAQLRRTIEC-CRTNLC                        AKL-3/CR
MEHHVRTC------IPKVELVPAGKPFYCLSSED--LRNTHYC-CRTDYC                       AKL-4/CR
KVIHNSRTC-----IAEIDLIPRDRPFVCAPSSKTGSVTTTYC-CNQDHC                       AKL-5/CR
IEIVKQGCWLDDINCYDRTD-----CVEKKDSPE--VYFCCCEGNMC                          ActR-II/CR
IELVKKGCWLDDFNCYDRQE-----CVAATEENPQ--VYFCCCEGNFC                         ActR-IIB/CR
NITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDEC                       TβR-II/CR
PEISHFGC-MDEKSVTDETEFHDTAAKVCTNNTKDPHATVWICCDKGNFC                       DAF-1/CR
```

FIG. 6

	ALK-2	ALK-3	ALK-4	ALK-5	ActR-II	ActR-IIB	TβR-II	daf-1	
	79	60	61	63	40	40	37	39	ALK-1
		63	64	65	41	39	37	39	ALK-2
			63	65	41	38	37	39	ALK-3
				90	41	40	39	42	ALK-4
					42	40	41	43	ALK-5
						78	48	35	ActR-II
							47	32	ActR-IIB
								34	TβR-II

FIG. 7

ALK-3
ALK-6
TβR-I/ALK-5
ALK-4
ALK-1
TβR-I/Tsk-7L/ALK-2/SKR1
ActR-II
ActR-IIB
TβR-II
Daf-1

FIG. 8

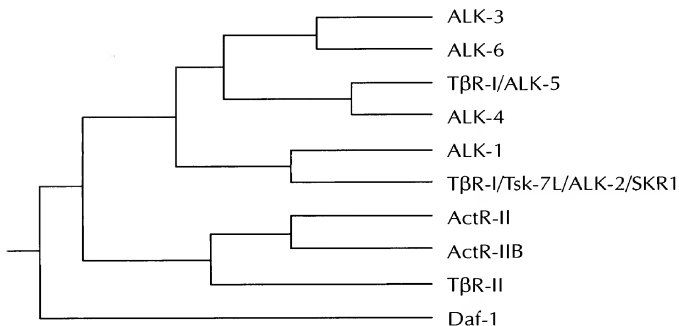| FLAG-Smad5	-	+	+	+
c.a. ALK1-HA	-	-	+	-
c.a. ALK5-HA	-	-	-	+

IP : anti-FLAG
Blot : anti-phosphoserine

IP : anti-FLAG
Blot : anti-FLAG

IP : (-)
Blot : anti-HA